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New records of *Phallales* from Paraguay

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Abstract—Four phalloid fungal species collected in the Central Department are reported for the first time from Paraguay: *Clathrus columnatus*, *Itajahya galericulata*, *I. rosea,* and *Mutinus argentinus*. Macro- and microscopic features are described for each species and photographs of fresh basidiomata are also provided. Additional comments address species distribution and taxonomy.

Key words—*Clathraceae*, gasteroid fungi, neotropical fungal diversity, *Phallaceae*, stinkhorns

Introduction

Gasteromycetes Fr. is no longer recognized as a formal class (Hibbett et al. 1997), and some of its members have been accommodated into the subclass *Phallomycetidae* K. Hosaka et al. within the orders *Gomphales* Jülich, *Phallales* E. Fisch., *Hysterangiales* K. Hosaka & Castellano, and *Geastrales* K. Hosaka & Castellano (Hosaka et al. 2006, Hibbett et al. 2007).

Within the *Phallales* spores are dispersed by insects, especially *Diptera* (Hibbett et al., 1997); *Phallaceae* Corda is a well defined family that embraces the commonly known "stinkhorns," which are characterized primarily by a simple hollow pseudostipe and a slimy spore mass (glebal mass) usually supported by a campanulate receptacle or spread over the pseudostipe surface (Burt 1896, Lloyd 1909, Cunningham 1944, Zeller 1949, Calonge 1998).

Several genera have been included in *Phallaceae*. Cunningham (1944) accepted seven genera: *Aporophallus* Möller, *Dictyophora* Desv., *Floccomutinus* Henn., *Itajahya* Möller, *Mutinus* Fr., *Phallus* L., and *Staheliomyces* E. Fisch. Zeller (1949) included another three: *Echinophallus* Henn., *Jansia* Penz., and *Xylophallus* (Schltdl.) E. Fisch. More recently, Kreisel (1996) treated *Aporophallus*, *Dictyophora*, and *Itajahya* as synonymous with *Phallus*, a taxonomic treatment that has been accepted for many years (Calonge 2005).

Although phylogenetic studies based on molecular data currently support *Dictyophora* within *Phallus* (Hosaka et al. 2006, Trierveiler-Pereira et al. 2014), however, they also support an independent generic status for *Itajahya* with two species: *I. galericulata* and *I. rosea*. *Itajahya* can be separated morphologically from other phalloid genera by the presence of a discoid structure (calyptra) that surrounds the receptacle apex and the morphology of the receptacle (Cabral et al. 2012, Marincowitz et al. 2015). The absence of receptacle and veil separate *Mutinus* species where the mucilaginous glebal mass spreads directly on the pseudostipe surface (Calonge 1998).

For many years, *Clathraceae* Chevall. has been regarded as separate from *Phallaceae* (e.g., Hawksworth et al. 1995: 581). Dring (1980) characterized taxa in the *Clathraceae* by basidiomes bearing the mucilaginous gleba on the inner surface of the receptacle, which may be lattice-shaped or consist of several columns. When the gleba is exposed to the exterior, then it is located on the surface of an apical lattice or column cluster. Although molecular studies support *Clathraceae* as a monophyletic group worthy of recognition at family level (Hosaka et al. 2006, Trierveiler-Pereira et al. 2014), *Phallaceae* sensu stricto and *Clathraceae* are still frequently treated as a single family, *Phallaceae* sensu lato (Kirk et al. 2001, 2008).

Mycological research in Paraguay was initiated by Spegazzini (1884, 1888, 1891), who reported 12 species from the country. More recent studies have been carried out by Campi and collaborators (Campi et al. 2012, 2015; Campi & Maubet 2015a,b). Here we report four new records of phalloid species from Paraguay: *Clathrus columnatus* (*Clathraceae*) and *Itajahya galericulata*, *I. rosea*, and *Mutinus argentinus* (*Phallaceae*).

Materials & methods

Specimens were collected in the Central Department, Paraguay, on the Universidad Nacional de Asunción campus and in the Asunción urban area. All specimens are deposited at the herbarium of the Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Asunción, Paraguay (FACEN).

Description of macroscopic features, as basidiomata size, shape and colors, were based on fresh specimens. For the microscopic analysis portions of the basidiomata were mounted in 5% KOH and examined with a Carl Zeiss compound microscope. Anatomical structures were dyed with phloxine and Congo red.

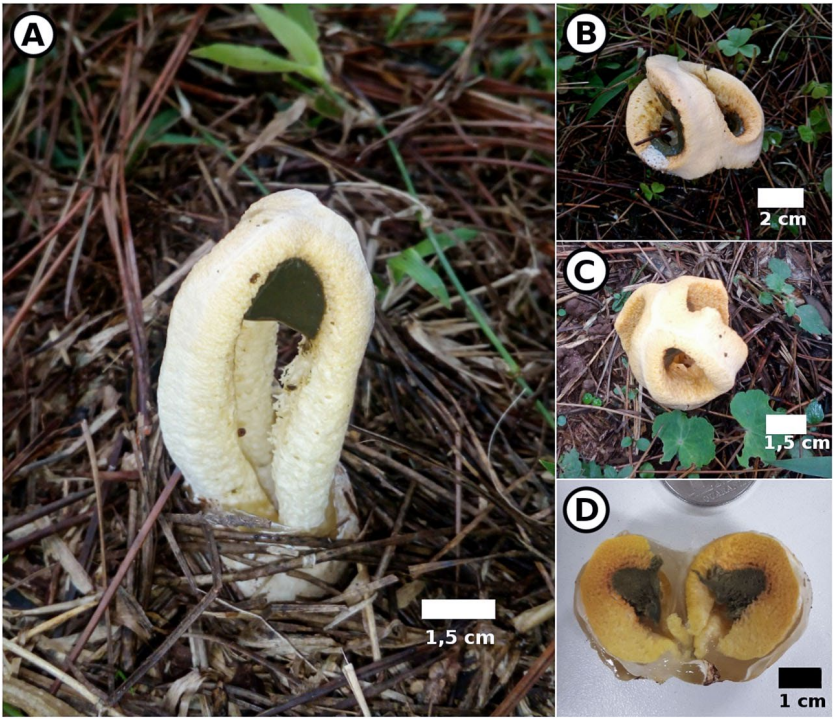

Fig 1. *Clathrus columnatus*.
A-C. Basidioma in situ growing near *Pinus*. D. Immature basidioma in transverse section.

Taxonomy

Clathrus columnatus Bosc, Mag. Gesell. Naturf. Freunde, Berlin 5: 85, 1811. Fig. 1

Immature basidiomata epigeous, globose to ovoid, 3.2–3.7 × 2.5–2.7 cm, attached to the substrate by rhizomorphs ≤7 cm long. Peridium of two layers, the outer membranous, <0.1 cm thick, whitish, smooth, covering the inner gelatinous layer, colorless, thickening (≤4 cm thick) towards the apex until only the exoperidium remains covering the columns of the mature basidioma. Dehiscing by irregular tears from the apex or occasionally

circumferentially. Expanded basidiome 6.8–10.3 cm high, whitish at the base, becoming yellowish to orange or reddish towards the apex, divided into 3–4 columns of 1.4–1.9 cm diam., spongy, free at the base, attached together at the apical zone by a transverse column. Gleba confined in the inner portion of the apex, olivaceous and mucilaginous, odor fetid.

Basidiospores 4–5 × 1.3–2 µm, cylindrical, hyaline to greenish in KOH, congophilic, with 1–2 internal guttules at the ends, thin-walled, smooth. Columns composed of pseudoparenchymatous, irregularly rounded cells, 16–35 µm diam. Exoperidium composed of septate hyphae, 4.5–7.5 µm diam., thin-walled, branched; the inner gelatinous layer composed of thin-walled hyphae, (2–)3–4 µm diam., hyaline; septa with clamp connections.

Ecology & distribution—solitary or in small groups, appearing soon after rain in cold temperatures on soil among litter in a *Pinus elliottii* Engelm. plantation in an urban setting.

Specimens examined—**PARAGUAY**. **Central Department**, **San Lorenzo,** 25°20′13″S 57°31′33″W, 26.V.2016, leg. Y. Maubet N° 16 (FACEN 3343).

Distribution in South America—Brazil (Trierveiler-Pereira & Baseia 2009, Magnago et al. 2013) and Chile (Sandoval-Leiva et al. 2014). This is the first record of *C. columnatus* from Paraguay.

Comments—Dring (1980) noted that a variety of forms can be found with respect to the number of arms and the general shape of the receptacle. In North American specimens, a transverse arm connects the vertical arms, as in our material. Dring (1980) also observed that the type description of *Clathrus australis* Speg. agrees with the type description of *C. columnatus* except for the presence of a fifth column. Unfortunately, as the Argentinean type material *C. australis* could not be found, some taxonomic doubt remains regarding the separation of the two species. Spegazzini (1887a) stated that he also had samples of *C. australis* from Paraguay, but they were in very bad condition. Spegazzini (1887b) later retained both *C. columnatus* and *C. australis* from Brazil as independent species.

Sandoval-Leiva et al. (2014) and Magnago et al. (2013) characterize the species as having 2–5 robust spongy reddish to orange columns free at the base and fused at the apex and a gleba that is spread on the internal portion of the columns and not confined to a glebifer. Based on the description and illustrations presented by previous authors (Dring 1980, Trierveiler-Pereira 2015) we conclude that our material represents *C. columnatus*.

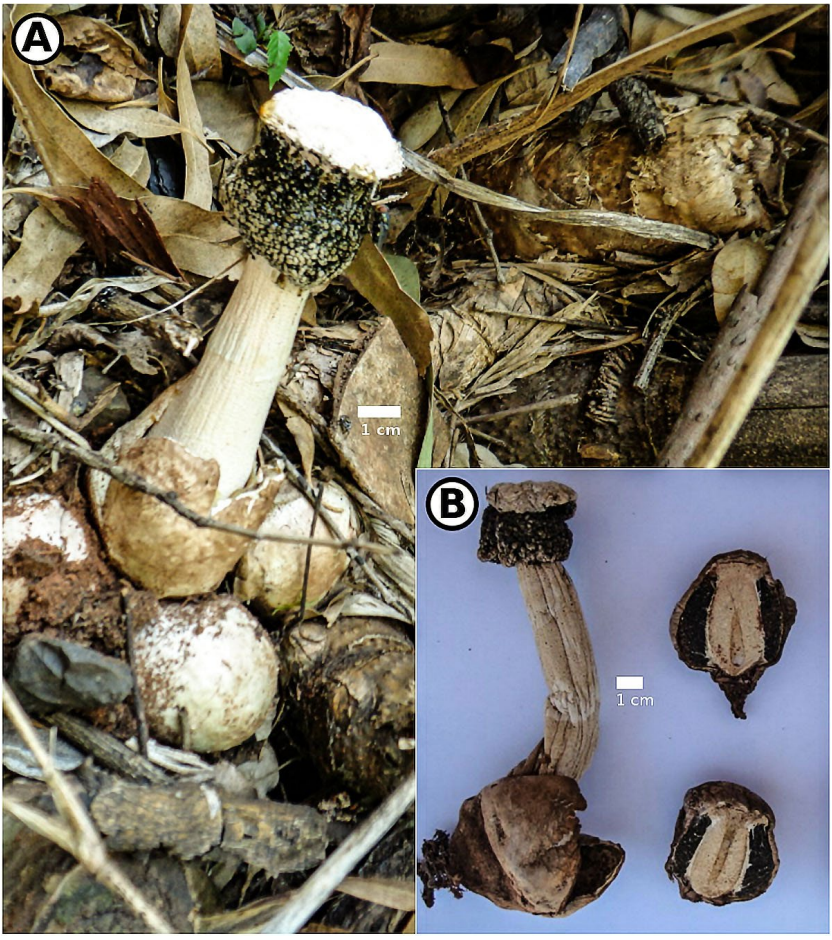

Fig. 2. *Itajahya galericulata*. A. Basidioma in situ growing near *Guadua*. B. Expanded basidioma and immature stage in transverse section.

Itajahya galericulata Möller, Bras. Pilzbl.: 79, 148, 1895. Fig. 2

Immature basidiomata epigeous, subglobose to globose, 4–5 × 3.5–4 cm, exoperidium ochre to dun, slightly leathery at the apex. Basal mycelial cord thick, 3–5 cm long and 0.2–0.4 cm thick. In transverse section, the peridium has four distinguishable layers with the continuous mucilaginous layer located between the other layers between the peridium and gleba. Expanded basidioma phalloid in shape. Stipe white, hollow, spongy and

externally longitudinally striated, 13.5–20 cm high × 2–4 cm diam., tapering towards the base. Receptacle cylindrical with grooved surface, up to 3.5 × 2.5 cm, covered with the mucilaginous gleba. Gleba deliquescent, dark green, odor putrid; calyptra at the receptacle apex white, discoid, flattened, 2–3 cm in diam., spongy, forming a sterile platform above the receptacle.

Basidiospores 5–6 × 2–3 μm, cylindrical, hyaline to slightly light green in KOH, thin-walled, smooth. Receptacle hyphae pseudoparenchymatous, globose to cylindrical, 400–100 × 20–45 μm; calyptra hyphae pseudoparenchymatous (globose to cylindrical, 33–70 × 24–34 μm) and thin-walled (septate, clamped, 2–13 μm diam.). Mycelial cord hyphae thin-walled, septate, clamped, 3–5 μm diam.

Ecology & distribution—Gregarious, fruiting soon after heavy rain during summer on soil among fallen litter and undergrowth in an urban area.

Specimens examined—**PARAGUAY**. **Central Department**, **Asunción**, 25°18′23″S 57°35′41″W, 21.I.2014, leg. Y. Maubet N° 38 (FACEN 3424).

Distribution in South America—Brazil (Möller 1895, Rick 1961), Argentina (Spegazzini 1927, Fries 1909, Wright 1949, Ruíz Leal 1954, Domínguez de Toledo 1995, Hernández Caffot et al. 2015), and Bolivia (Rocabado et al. 2007). This is the first record of *I. galericulata* from Paraguay.

Comments—Möller (1895) stated that in South America *Itajahya* basidiomata are very variable in size and development at the edge of the receptacle and calyptra, unlike specimens found in North America, where they appear more stable (Long & Stouffer 1943). Domínguez de Toledo (1995) observed that in its "egg phase" the *I. galericulata* peridium consists of four layers around the gleba: two firm layers surrounding a mucilaginous and continuous layer, the same arrangement as we found in our specimen. We also observed longitudinal stripes in the stipe, a remarkable character that has not been noted in the literature. The morphological feature separating *I. galericulata* from *I. rosea* is the color of the pseudostipe, which is pinkish in the latter.

Marincowitz et al. (2015) reported an association between *I. galericulata* and the tree species *Jacaranda mimosifolia* D. Don in Pretoria, South Africa. In Brazil, Bolivia, and Paraguay, specimens were collected in pastures of urban areas in accordance with the ecology noted by Domínguez Toledo (1995) and Hernández Caffot et al. (2015), who described *I. galericulata* growing in compacted soils with low grasses during summer and autumn.

Fig. 3. *Itajahya rosea*. A. Basidioma in situ growing among grass. B. Immature stage in transverse section and expanded basidioma.

Itajahya rosea (Delile) E. Fisch., Ber. Deutsch. Bot. Ges. 47: 294, 1929 Fig. 3

Immature basidiomata epigeous, subglobose to globose, 1.4–1.7 × 3–3.4 cm, exoperidium ochre to dun, slightly leathery at the apex. In transverse section, it is possible to observe that the pseudostipe is pink and surrounded by three layers: outer layer 0.3 cm thick, firm, white to beige, crackled; middle layer 0.8 cm thick, mucilaginous, aqueous, hyaline; inner layer >0.1 cm thick, whitish, dry, covering the green gleba. Expanded basidioma Stipe white, hollow, fibrous, 9.5–14.2 × 1.6–1.8 cm, slightly tapering towards the base. Receptaculum conic with reticulated surface, 2 × 1 cm, covered with gleba. Gleba mucilaginous, deliquescent, dark green, odor fetid; calyptra at the apex of the receptaculum white, discoid, flattened, 1.1–1.9 cm in diam., forming a sterile platform above the receptacle.

Basidiospores 4 × 2 µm, cylindrical, hyaline, slightly green, thin-walled, smooth. Stipe hyphae pseudoparenchymatous, globose, 15–25(–35) ×

22–35(–46) µm. Receptacle hyphae pseudoparenchymatous, globose to cylindrical, 12–23(–26) × 8–14(–16) µm; calyptra hyphae thin-walled, septate, clamped, 3–5(–6) µm diam.

Ecology & distribution—Gregarious, appearing soon after heavy rain during summer.

Specimens examined—**PARAGUAY. Central Department, San Lorenzo,** Campus Universitario, 26°20′08″S 57°31′14″W, I.2016, leg. Y. Maubet N° 002 (FACEN 3338).

Distribution in South America—Northeastern Brazil (Ottoni et al. 2010, Cabral et al. 2012). This is the first record of *I. rosea* from Paraguay.

Comments—According to Ottoni et al. (2010), the most relevant characteristics for the taxonomical determination of *I. rosea* is the presence of a calyptra at the apex of the receptacle and the pinkish pseudostipe. The species is morphologically very close to *I. galericulata* (see comments above). The peridium of the immature basidiome is another important feature separating the species: three-layered in *I. rosea* and four-layered in *I. galericulata*.

Moreno et al. (2013) recognized *I. rosea* as a rare species characteristic of desert and semiarid regions, as reported from Yemen (Kreisel & Al-Fatimi 2008) and Northeastern Brazil (Ottoni et al. 2010). However, our specimen was found in eastern Paraguay characterized by a subtropical climate with the rainy season occurring in the summer.

Future molecular research is needed to separate the two morphologically similar species.

Mutinus argentinus Speg., Anal. Soc. Cient. Argent. 24: 62, 1887. Fig. 4

Immature basidiomata epigeous, ellipsoid to ovoid, 3.0 × 1.8 cm, whitish, with basal rhizomorphs 3–6 cm long, fibrous, strongly attached to the substratum. In transverse section releasing an odorless translucent liquid; three layers surrounding the pseudostipe: the outer layer 0.1 cm thick, whitish to cream, covered with brownish fibers; medium layer 0.4 cm thick, wet, sticky, hyaline; inner layer >0.1 cm, very thin, whitish, enclosing the gleba. Expanded basidiome Stipe 6.6 × 0.9 cm, spongy, whitish at the base, becoming pinkish towards the apex, and finally bright red to maroon at the apex, apex with a small pore. Gleba mucilaginous, olive green, covering the stipe apex.

Basidiospores 5–7 × 2–3 µm, cylindrical, hyaline, slightly green, thin-walled, smooth. Stipe hyphae pseudoparenchymatous, globose, 35–50 × 50–70 µm, Congo-Red negative. Glebal region hyphae pseudoparenchymatous, cylindrical, spherical to irregular in shape, 30–50 × 25–30 µm, pinkish.

Fig. 4. *Mutinus argentinus*.
A. Basidiome in situ on dead branch, among leaves. B. Immature stage on decaying wood and expanded basidiome and immature stage in transverse section.

Ecology & distribution—Growing solitary or in small groups of a few basidiomata on sandy soil among decaying fallen litter and wood debris.

Specimens examined—**PARAGUAY. Central Department**, San Lorenzo, Campus Universitário, 26°20′08″S 57°31′14″W, 23.III.2014, leg. M. Campi N° 45 (FACEN 3356).

Distribution in South America—Argentina (Spegazzini 1887a, 1906; Wright 1949) and Brazil (Meijer 2006, Magnago et al. 2013). This is the first record of *M. argentinus* from Paraguay.

Comments—Magnago et al. (2013) discussed the similarity of *M. argentinus* and *M. bambusinus* (Zoll.) E. Fisch., two species that have been treated as synonyms by several authors. Dring & Rose (1977) presented the morphological differences between the two species: *M. bambusinus* produces a larger fertile portion that is strongly pigmented and with a sterile tip, while *M. argentinus* lacks the marked sterile tip. The species is also depicted by Trierveiler-Pereira (2015). Based on this information and the provisional *Mutinus* key by Silva et al. (2015), we conclude that our specimen represents *M. argentinus*.

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